THE ENERGY OF UREA SYNTHESIS. II. THE EFFECT OF VARYING HYDROGEN ION CONCENTRATION WITH DIFFERENT METABOLITES

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In a previous communication[1] we have reported that an increased oxygen consumption can be observed accompanying the synthesis of urea by liver slices from ammonium bicarbonate. This observation is now confirmed, and the results of additional experiments are presented which elucidate to some extent some of the general features of the mechanism of the transfer of energy in this and probably other coupled reactions.

The technique employed here was similar to that in the experiments reported previously, except that the vessels used were two and one-half times smaller, and the liver slices were placed in 3 cc. instead of 15 cc. of fluid. The composition of the Ringers solution was varied according to the pH desired. The hydrogen ion concentrations were measured colorimetrically before and after each experiment. The ornithine and the ammonium chloride, wherever it was used, were first brought to pH 7.4 with sodium hydroxide. Q_{O_2} is the number of cubic millimeters of O_2 at standard temperature and pressure used per milligram of dry weight of tissue (dried at 100°C.) per hour. Q_{urea} is the number of cubic millimeters of CO_2, at standard temperature and pressure, liberated at the end of the experiment at pH 5.0 by urease per milligram of dry weight of tissue per hour.

Some of the results obtained are given in the table. They show that with no metabolite added or with glucose, at pH 7.4 and 8.4 one molecule of additional oxygen is used for every molecule of urea synthesized. With dl lactate and with succinate at pH 8.4 the results were the same. On the other hand, at pH 7.4 with the latter two metabolites there was much less additional oxygen used in the synthesis of urea. These observations resolve the apparent discrepancy between our previous observations and those of Krebs and Henseleit.[2]

The latter authors worked for the most part at pH 7.4. The one experiment whose protocoll on oxygen consumption is given was carried out at this pH with dl lactate as metabolite. The Ringers solution was the same as in experiments 1 and 2 above, and a gas mixture containing 5 per cent CO_2 and 95 per cent O_2 was used. The CO_2 was not absorbed and so maintained the hydrogen ion concentration of the Ringers solution at pH 7.4. In our experiments the gas was pure O_2, and the CO_2 was continually ab-

sorbed. As a result, in our previous experiments, a large fraction of the bicarbonate was decomposed, producing an alkaline reaction until finally a balance was attained between the CO_2 lost by the solution and that produced by the respiration of the tissue. We were, of course, aware of this induced alkalinity, but these conditions were preferred in those exploratory experiments because they permitted us to observe the rate of respiration throughout the experiment; and because larger amounts of urea are synthesized under these conditions than when the pH was maintained at 7.4 by a phosphate buffer. Warburg has shown that between pH 6.5 and 8.5 the respiration of liver slices is not affected by change in hydrogen ion concentration,[3] which our own experience confirmed.

In order to obviate a lengthy discussion of the different hypotheses which might be invoked to explain these results, we shall describe briefly the results of some experiments in a different field which bear directly upon the question of the mechanism of the transfer of energy in biological coupled reactions.* In these experiments it was found that in order to obtain under anaerobic conditions the synthesis of lactate from pyruvate through the oxidation of formate by toluene treated bacteria, it was necessary to add a small amount of some substance (usually a dye) which is capable of oxidation or reduction by both the formic acid and lactic acid dehydrogenase mechanisms. In the absence of the dye the formic acid was not oxidized and the pyruvic acid was not reduced. Similar results were obtained with the formation of succinic acid from fumaric acid at the expense of the oxidation of lactic acid to pyruvic acid. The transfer of energy here was effected by the dye, which passed in the reduced state from the locus where, for example, formic acid was oxidized and the dye reduced, to the center where, by oxidation of the dye, pyruvic acid was reduced to lactic acid.

The simplest hypothesis accounting for all of the facts at present seems to us as follows: The coupled reaction in which urea is synthesized from ammonia is essentially the same with all the metabolites used, at pH 7.4, as well as at pH 8.4; because in all cases the synthesis of urea is accelerated by ornithine; and because with lactate and succinate at pH 8.4 the ratio of increased oxygen used to urea synthesized is the same as with glucose or no added metabolite at pH 7.4 and pH 8.4. Here the energy produced is nearly 10 times the amount necessary, and is derived from specific oxidations and not from the general respiration of the cell. The synthesis, i.e., the transfer of energy occurs only at certain specific centers. This is shown by the absence of any correlation between the total rate of metabolism, in the absence of urea synthesis, and the amount of urea synthesized when ammonia and ornithine are added. This is further sup-

* These experiments were carried out with Mr. H. F. Schott in this laboratory, and will be submitted for publication shortly.

RELATION BETWEEN ADDITIONAL OXYGEN CONSUMPTION AND UREA SYNTHESIS BY LIVER TISSUE IN RINGERS SOLUTIONS AT DIFFERENT HYDROGEN ION CONCENTRATIONS AND WITH DIFFERENT METABOLITES

EXPERIMENT NO.	SOURCE OF AMMONIA	TYPE OF RINGERS SOLUTION USED	pH	ORNITHINE CONCENTRATION	AMMONIA CONCENTRATION	DRY WT. OF TISSUE	DURATION OF EXPERIMENT	O_2 CONSUMPTION	UREA FOUND	ΔQ_{O_2}	ΔQurea	$\frac{\Delta Q_{O_2}}{\Delta Q\text{urea}}$
				MG. %	MG. %	MG.	MINUTES	Q_{O_2}	Qurea			
				10	..	13.4		9.0	0.3			
7	NH_4Cl	0.2% Glucose in phosphate Ringers	7.3				87			2.6	2.7	1.0
				10	25	12.4		11.6	3.0			
				10	25	20.6		10.7	2.1			
8	NH_4Cl	0.2% Glucose in phosphate Ringers	7.4				87			2.3	2.0	1.2
				10	..	20.2		8.4	0.1			
				10	25	12.9		16.2	6.2			
1	NH_4HCO_3	Bicarbonate Ringers	8.4				85			5.7	5.9	1.0
				10	..	13.0		10.5	0.3			
				10	25	13.7		16.0	5.8			
2	NH_4HCO_3	Bicarbonate Ringers	8.4				85			5.6	5.3	1.05
				10	..	14.3		10.4	0.5			
				10	..	15.8		11.3	0.3			
21	NH_4Cl	Phosphate Ringers	7.4				85			1.4	1.6	0.9
				10	25	13.9		12.7	1.9			
				10	..	13.7		10.2	0.2			
22	NH_4Cl	Phosphate Ringers	7.4				85			1.6	1.6	1.0
				10	25	13.6		11.8	1.8			
				10	25	14.5		16.6	5.2			
19	NH_4Cl	Bicarbonate Ringers	8.2				91			4.1	4.7	0.9
				10	..	14.4		12.5	0.5			

				10	25	14.9		16.5	5.3			
20	NH_4Cl	Bicarbonate Ringers	8.2				91			3.6	4.6	0.8
				10	..	13.2		12.9	0.7			
				10	25	11.9		16.1	3.3			
10	NH_4Cl	0.2% dl lactate in phosphate Ringers	7.4				95			1.5	2.8	0.5
				10	..	11.7		14.6	0.5			
				10	25	9.9		17.1	3.8			
11	NH_4Cl	0.2% dl lactate in phosphate Ringers	7.4							1.1	3.3	0.3
						10.1	95	16.0	0.6			
				10	..	10.3		15.0	0.0			
12	NH_4Cl	0.2% dl lactate in bicarbonate Ringers	8.4				89			2.5	2.5	1.0
				10	25	8.0		17.5	2.5			
				10	..	10.7		14.6	0.0			
13	NH_4Cl	0.2% dl lactate in bicarbonate Ringers	8.4				89			2.1	2.2	1.0
				10	25	10.4		16.7	2.2			
				10	25	15.8		16.0	2.1			
23	NH_4Cl	$^1/_{90}$ molal succinate in phosphate Ringers	7.4				98			−0.5	1.9	0.0
				10	..	14.2		16.5	0.3			
				10	25	14.3		16.9	2.1			
24	NH_4Cl	$^1/_{90}$ molal succinate in phosphate Ringers	7.4				98			−0.1	1.9	0.0
				10	..	14.4		17.0	0.3			
				10	25	15.7		20.3	3.2			
17	NH_4Cl	$^1/_{90}$ molal succinate in bicarbonate Ringers	8.3				78			2.7	2.7	1.0
				10	..	16.0		17.6	0.5			
				10	25	14.7		21.5	3.6			
18	NH_4Cl	$^1/_{90}$ molal succinate in bicarbonate Ringers	8.3				78			3.0	3.1	1.0
				10	..	14.2		18.5	0.5			

ported by the experiments on the reductive formation of succinic acid and lactic acid mentioned above; and by the demonstration by Krebs and Henseleit that urea synthesis is obtained only with intact cells; when the structure of the liver cells is destroyed by grinding, though respiration still occurs there is no longer any synthesis of urea. This evidence for the existence of specific centers also strengthens the first point that the essential mechanism of the coupled reaction is the same under all conditions.

It follows therefore that with lactate and succinate at pH 7.4 (but not at pH 8.4) other reactions requiring energy are in progress. In these the energy supplied to them is greatly in excess of that required. When ornithine and ammonia are added some of this surplus energy, which is possibly used in other reactions or which passes off as heat, is used in the synthesis of urea. The result is that under these conditions the increased oxygen consumption accompanying the synthesis of urea is less than under other conditions, e.g., at pH 8.4 or with glucose instead of lactate or succinate, when the increased oxygen consumption is proportional to the amount of urea synthesized.

The synthesis of glycogen suggests itself as an obvious possibility for this first-mentioned reaction requiring energy upon which the synthesis of urea is superimposed. It has been shown that both lactate and succinate may be converted to glycogen in the liver.[4,5] In the case of the synthesis of glycogen from lactate it is well known that more energy is produced than the theoretical minimum necessary. It is probable that a similar state of affairs exists in the synthesis of glycogen from succinate.

It is necessary to the hypothesis advanced above that the metabolite added to the Ringers solution, particularly the lactate and succinate, be used. The results show that, in the absence of ammonia, at all the hydrogen ion concentrations tried, the rate of respiration with the latter two metabolites was much higher than when no metabolite, or when glucose was added.

It is clear from the above data that the total energy change is not governed by the energy requirements of the reaction in which ΔF is positive. The total quantity of energy produced is much greater than the necessary minimum amount computed from solely thermodynamical considerations. It is probable that this is a very common, if not a universal feature of biological coupled reactions. The production of a large amount of excess energy in the synthesis of glycogen from lactic acid may be cited as another example. The 1:1 ratio between additional oxygen used and urea synthesized, suggests that the factor governing the total energy change here is a stoichiometrical one, the consumption of a molecule of oxygen really representing the production of a molecule of CO_2 at a locus in the cell, and in such a state that it can be combined with ammonia and the other components necessary for the formation of urea.

Summary.—Experimental results are presented which indicate (1) that one molecule of oxygen is used for every molecule of urea synthesized; (2) that the total energy change is governed by the stoichiometrical rather than by the energy requirements of the reaction in which the free energy change (ΔF) is positive; (3) the existence of specific centers and specific mechanisms in the cell whereby energy is transferred from specific energy liberating reactions (i.e., not the general respiration of the cell) to the energy absorbing reaction; and (4) that this specificity does not exclude mechanisms whereby the energy produced by an energy liberating reaction may be shared by more than one energy absorbing reaction.

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[2] Krebs, H. A., and Henseleit, K., *Zeit. physiol. Chem.*, **210**, 33 (1932).

[3] Warburg, O., *Biochem. Z.*, **143**, 317 (1923).

[4] Himwich, H. E., Koskoff, Y. D., and Nahum, L. H., *J. Biol. Chem.*, **85**, 571 (1930); Bayo, C. Pi-Suñer, and Pi, J. F., *Biochem. Z.*, **242**, 306 (1931).

[5] Ponsford, A. P., and Smedley-Maclean, I., *Biochem. J.*, **26**, 1340 (1932).

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*ON THE TEMPERATURE-REGULATORY FUNCTION OF "SPONTANEOUS" ACTIVITY IN THE MOUSE**

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When every effort is made to eliminate excitation in the external environment of a young mouse, movements still occur—the so-called "spontaneous" movements. These movements do not occur haphazardly, but with a definite form, namely, in regularly recurring periods of movement alternating with periods of quiet. The periods of activity and of quiescence are of statistically constant length, having a mean probable error, as percentage of the mean, of 5 per cent for activity, of 10 per cent for quiescence, at 21 ± 0.01°C. (Stier, 1930). The mice are still poikilothermic at the age of two days; the frequency of occurrence of activity cycles increases with temperature in the manner of a chemical reaction velocity (μ, in the Arrhenius equation $\ln K \propto \mu/RT$, was found to be about 25,300 calories; cf. Stier, 1930). The duration of a period of activity, however, decreases as the body temperature increases, e.g., at 16.5°C. the average duration of a single activity period was 0.7 min., at 29.5°C. the average duration was 0.3 min.

The average duration of activity periods times the number of periods per hour yields a quantity which should be roughly proportional to the rate at which heat is produced by muscular movements of the young mouse.